# The resistance profile of Acinetobacter baumannii strains isolated from the Aberdeen Royal Infirmary

**Table 1**
*Streptococcus* sp. isolates identified and their Tn*916*-like elements. Organisms were identified to species level according to the nearest match in GenBank and were then sorted into strains according to sequence differences in the *sod*(A) gene.

| Species ID | No. of isolates | Elements present (no. of isolates) | Excision (no. of isolates)[a] |
|---|---|---|---|
| *S. anginosus* | 1 | Tn*916* | y |
| *S. australis* I | 1 | Tn*6002* | n |
| *S. australis* II | 1 | Tn*6087* | y |
| *S. gordonii* | 1 | Tn*916* | y |
| *S. infantis* I | 3 | Tn*6002* (1), Tn*916* (2) | y |
| *S. infantis* II | 1 | Tn*916* | y |
| *S. infantis* III | 1 | Tn*916* | y |
| *S. mitis* | 1 | Tn*6002* | y |
| *S. oralis* I | 1 | Tn*6002* | y |
| *S. oralis* II | 1 | Tn*916* | y |
| *S. parasanguinis* I | 2 | Tn*6002* | y |
| *S. parasanguinis* II | 1 | Tn*916* | y |
| *S. salivarius* I[b] | 1 | Tn*3872* and Tn*916* | y |
| *S. salivarius* II | 6 | Tn*6002* | y (2)/n (4) |
| *S. salivarius* III | 12 | Tn*3872* (11), Tn*916* (1) | y (Tn*3872* ×6, Tn*916*)/n (Tn*3872* ×5) |
| *S. salivarius* IV | 3 | Tn*3872* | y |
| *S. salivarius* V | 1 | Tn*916* | n |
| *S. salivarius* VI | 1 | Tn*3872* | n |
| *S. salivarius* VII | 1 | Tn*916* | y |
| *S. salivarius* VIII | 1 | Tn*916* | n |
| *S. salivarius* IX | 1 | Tn*916* | y |
| *S. salivarius* X | 1 | Tn*916* | y |
| *S. sanguinis* I | 2 | Tn*3872* (1), Tn*6002* (1) | y (Tn*3872*)/n (Tn*6002*) |
| *S. sanguinis* II | 2 | Tn*3872* | y |
| *S. sanguinis* III | 1 | Tn*6002* | y |

[a] y, yes; n, no.
[b] Southern blot analysis of isolate *S. salivarius* I was found to contain three copies of *intTn*, suggesting that an integrase gene may be present but not associated with a Tn*916*-like element.

the composition of the indigenous microbiota at various body sites'.

*Competing interests*: None declared.

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## The resistance profile of *Acinetobacter baumannii* strains isolated from the Aberdeen Royal Infirmary

*Sir,*

The increase in carbapenem resistance in *Acinetobacter baumannii* is largely attributable to the Ambler class D β-lactamases, in particular enzymes related to OXA-23 and OXA-58. The purpose of this study was to analyse the resistance in *A. baumannii* strains isolated from Aberdeen Royal Infirmary (Aberdeen, UK) from 2006 to 2010.

Nine non-repetitive *A. baumannii* strains were chosen for this study. The strains were identified by polymerase chain reaction (PCR) of the $bla_{\text{OXA-51-like}}$ gene and by sequencing of the *rpoB* gene. Minimum inhibitory concentrations (MICs) were determined according to the guidelines of the British Society for Antimicrobial Chemotherapy (BSAC). The $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-40-like}}$, $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-58-like}}$ and $bla_{\text{OXA-143-like}}$ gene families were screened by multiplex PCR [1]. ISADC1 and OXA-23R primers were used for the detection of IS*Aba1* upstream of the $bla_{\text{OXA-23-like}}$ gene [1,2]. Aminoglycoside resistance genes were identified by multiplex PCR [3]. Primers for amplification of the $bla_{\text{ADC}}$ gene have been described previously [2], and primers FU (5′-GCG CCG TGA ATT CTT AAG TG-3′) and RU (5′-AGC CAT ACC TGG CAC ATC AT-3′) were used to amplify the intergenic region upstream of the $bla_{\text{ADC}}$ gene. PCR was performed for amplification of the quinolone resistance-determining regions (QRDRs) of the *gyrA* and *parC* genes [4], and gene fragments were sequenced for determining specific amino acid changes. PCR for detection of the class 1 integrase gene was performed as described previously [4].

A macrorestriction assay followed by pulsed-field gel electrophoresis (PFGE) was performed on all *A. baumannii* strains. Cluster analysis was performed by the unweighted pair-group method with mathematical averaging (UPGMA), and DNA relatedness was calculated using the band-based Dice coefficient with a tolerance setting of 1.5% band tolerance and 1.5% optimisation setting for the whole profile. Gel analysis was performed using BioNumerics v2.5 software (Applied Maths, Sint-Martens-Latem, Belgium). A value of ≥80% was chosen as the threshold for the establishment of clonal relatedness of the isolates.

*S1* nuclease (Promega, Southampton, UK) digestion using 10 U of enzyme with incubation at 37 °C for 45 min was performed for the PFGE plugs according to the manufacturer's instructions. Plasmid curing was performed using acriflavine and with an elevated temperature of incubation. The strains were serially subcultured for 14 days at 47 °C.

Two novel variants of the $bla_{\text{OXA-51-like}}$ gene were found (Table 1): strain 14 had serine-14 (TCT) of the $bla_{\text{OXA-180}}$ gene replaced by phenylalanine (TTT) and is now designated $bla_{\text{OXA-216}}$; and strain 6n had threonine-255 (ACA) of the $bla_{\text{OXA-78}}$ gene substituted by isoleucine (ATA) and is now designated $bla_{\text{OXA-217}}$. Isolates 10 and 10n had variants of $bla_{\text{OXA-65}}$ gene with synonymous mutations.

**Table 1**
Resistance profiles of *Acinetobacter baumannii* clinical strains.

| Strain no. | Source of isolation | Date of isolation | MIC (mg/L) | | | | | | *int1* | $bla_{ADC}$ | IS*Aba1*–$bla_{ADC}$ | $bla_{OXA-51-like}$ gene |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | MEM | IPM | CAZ | GEN | CIP | COL | | | | |
| 3 | Blood | 09/09/2006 | 1 | 0.5 | 64 | 256 | 32 | 0.25 | + | + | + | 66 |
| 10 | Blood | 08/06/2008 | 0.06 | 0.06 | 1 | 0.12 | 0.12 | 0.12 | – | – | – | 65 (variant) |
| 12 | Blood | 25/08/2008 | 16 | 16 | 64 | 8 | 32 | 0.5 | + | + | + | 66 |
| 14 | Blood | 21/09/2009 | 0.06 | 0.06 | 4 | 0.03 | 0.12 | 0.03 | – | + | – | 216 |
| 16 | Sputum | 20/02/2010 | 0.5 | 0.5 | 4 | 0.12 | 0.12 | 0.12 | – | + | – | 51 |
| 3n | Blood | 27/04/2006 | 0.5 | 0.25 | 2 | 0.03 | 0.12 | 0.12 | – | + | – | 64 |
| 6n | Blood | 17/07/2007 | 1 | 0.5 | 8 | 0.12 | 0.5 | 0.5 | – | + | – | 217 |
| 10n | Blood | 22/10/2008 | 0.06 | 0.06 | 1 | 0.03 | 0.12 | 0.12 | – | + | – | 65 (variant) |
| 14n | Blood | 14/09/2009 | 0.5 | 0.25 | 8 | 0.12 | 0.5 | 0.12 | – | + | – | 89 |

MIC, minimum inhibitory concentration; MEM, meropenem; IPM, imipenem; CAZ, ceftazidime; GEN, gentamicin; CIP, ciprofloxacin; COL, colistin.

Strains 3 and 12 possessed the *aac*(3′)-*Ia* gene conferring gentamicin resistance and had high ceftazidime MICs owing to the presence of IS*Aba1* upstream of the $bla_{ADC}$ gene. All of the remaining strains (except 10) had the $bla_{ADC}$ gene without IS*Aba1* upstream and thus were susceptible to ceftazidime (Table 1). Strain 10 completely lacked the $bla_{ADC}$ gene. All of the isolates were susceptible to colistin.

Strains 3 and 12 had amino acid changes at position 83 of GyrA (serine83 → leucine) and position 80 of ParC (serine80 → leucine) conferring ciprofloxacin resistance. Both strains possessed integrase genes and sequencing confirmed the presence of putative glucose dehydrogenase precursor that could be responsible for catabolism of glucose by oxidation.

The PFGE profiles of the strains showed that most were not clonally related as they had <80% similarity (data not shown). PCR for insertions causing disruption of c*arO* (29 kDa outer membrane protein) was not detected for any of the strains. PFGE analysis revealed that strains 3 and 12 had 83% similarity; strain 3, isolated in the year 2006, was negative for the IS*Aba1*–$bla_{OXA-23}$ gene, whereas strain 12, isolated in the year 2008, was positive and was resistant to imipenem and meropenem (Table 1). Strains 3 and 12 had an identical $bla_{OXA-51-like}$ allele 1 corresponding to sequence group 1. In addition, strain 12 had the $bla_{OXA-23}$ clone 1 allele, which belongs to European clone II. This indicates the *A. baumannii* is actively acquiring resistance genes, probably through plasmid transfer, although *S1* nuclease digestion and plasmid extraction procedures did not detect any plasmids, and elimination studies with acriflavine did not remove the resistance determinants. This suggests that if $bla_{OXA-23}$ was plasmid borne, it is now integrated in the host chromosome of strain 12 endowing it with a stable mechanism of carbapenem resistance.

These results show that the clinical situation in the hospital in Aberdeen is in a state of flux. New variant strains are emerging and, most importantly, a carbapenem-sensitive strain has become resistant through acquisition of the $bla_{OXA-23}$ gene with an IS*Aba1* element upstream that carries a promoter allowing expression of the β-lactamase. The $bla_{OXA-23}$ gene was first found in Scotland more than 20 years ago and it has remained the sole mechanism of carbapenem resistance until this point [5].

## Nucleotide accession numbers

The $bla_{OXA-216}$ and $bla_{OXA-217}$ genes have been deposited in GenBank under the accession nos. FR865168 and JN603240, respectively.

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